

P/ ENT COOPERATION TREA^T

From the INTERNATIONAL BUREAU

PCT**NOTIFICATION OF ELECTION**

(PCT Rule 61.2)

To:

Commissioner
 US Department of Commerce
 United States Patent and Trademark
 Office, PCT
 2011 South Clark Place Room
 CP2/5C24
 Arlington, VA 22202
 ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 05 February 2001 (05.02.01)	To: Commissioner US Department of Commerce United States Patent and Trademark Office, PCT 2011 South Clark Place Room CP2/5C24 Arlington, VA 22202 ETATS-UNIS D'AMERIQUE in its capacity as elected Office
International application No. PCT/SE00/01179	Applicant's or agent's file reference P15722PC/CA
International filing date (day/month/year) 07 June 2000 (07.06.00)	Priority date (day/month/year) 09 June 1999 (09.06.99)
Applicant OLMARKER, Kjell et al	

1. The designated Office is hereby notified of its election made:

in the demand filed with the International Preliminary Examining Authority on:

05 January 2001 (05.01.01)

in a notice effecting later election filed with the International Bureau on:

2. The election was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer R. E. Stoffel
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

Date of mailing (day/month/year) 15 February 2001 (15.02.01)	To: AWAPATENT AB Box 11394 S-404 28 Göteborg SUÈDE	
Applicant's or agent's file reference P15722PC/CA	IMPORTANT NOTIFICATION	
International application No. PCT/SE00/01179	International filing date (day/month/year) 07 June 2000 (07.06.00)	

1. The following indications appeared on record concerning: <input type="checkbox"/> the applicant <input type="checkbox"/> the inventor <input checked="" type="checkbox"/> the agent <input type="checkbox"/> the common representative				
Name and Address AWAPATENT AB Box 11394 S-404 28 Göteborg Sweden		State of Nationality		State of Residence
		Telephone No.		
		Facsimile No.		
		Teleprinter No.		

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning: <input type="checkbox"/> the person <input type="checkbox"/> the name <input type="checkbox"/> the address <input type="checkbox"/> the nationality <input type="checkbox"/> the residence				
Name and Address		State of Nationality		State of Residence
		Telephone No. +46 31 63 02 00		
		Facsimile No. +46 31 63 02 63		
		Teleprinter No.		

3. Further observations, if necessary: Please note that telephone and fax numbers of the agent have now been recorded.				
--	--	--	--	--

4. A copy of this notification has been sent to: <input checked="" type="checkbox"/> the receiving Office <input type="checkbox"/> the designated Offices concerned <input type="checkbox"/> the International Searching Authority <input checked="" type="checkbox"/> the elected Offices concerned <input checked="" type="checkbox"/> the International Preliminary Examining Authority <input type="checkbox"/> other:				
---	--	--	--	--

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer F. Baechler Telephone No.: (41-22) 338.83.38
---	---

P. PCT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)Date of mailing (day/month/year)
29 octobre 2001 (29.10.01)

To:

AWAPATENT AB
Box 11394
S-404 28 Göteborg
SUÈDEApplicant's or agent's file reference
P15722PC/CA

IMPORTANT NOTIFICATION

International application No.
PCT/SE00/01179International filing date (day/month/year)
07 juin 2000 (07.06.00)

1. The following indications appeared on record concerning:

the applicant the inventor the agent the common representative

Name and Address

State of Nationality

State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

the person the name the address the nationality the residence

Name and Address

A+SCIENCE INVEST AB
P.O. Box 3096
S-400 10 Göteborg
Sweden

State of Nationality

State of Residence

SE

SE

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

The above-identified company shall be added to the records as applicant for all designated States except the United States of America. RYDEVIK, Björn and ÖLMARKER, Kjell shall now be recorded as applicant/inventors for the US only.

4. A copy of this notification has been sent to:

the receiving Office
 the International Searching Authority
 the International Preliminary Examining Authority

the designated Offices concerned
 the elected Offices concerned
 other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Authorized officer

Beate GIFFO-SCHMITT

Facsimile No.: (41-22) 740.14.35

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

AWAPATENT AB
Box 11394
S-404 28 Göteborg
SUÈDEDate of mailing (day/month/year)
24 January 2001 (24.01.01)Applicant's or agent's file reference
P15722PC/CA

IMPORTANT NOTIFICATION

International application No.
PCT/SE00/01179International filing date (day/month/year)
07 June 2000 (07.06.00)

1. The following indications appeared on record concerning:

 the applicant the inventor the agent the common representative

Name and Address

GÖTEBORGS PATENTBYRÅ DAHLS AB
Sjöporten 4
S-417 64 Göteborg
Sweden

State of Nationality

State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

 the person the name the address the nationality the residence

Name and Address

AWAPATENT AB
Box 11394
S-404 28 Göteborg
Sweden

State of Nationality

State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

Please furnish new tel and fax number and power of attorneys from all applicants.

4. A copy of this notification has been sent to:

 the receiving Office
 the International Searching Authority
 the International Preliminary Examining Authority the designated Offices concerned
 the elected Offices concerned
 other:The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

G. Bähr

Telephone No.: (41-22) 338.83.38

INTERNATIONAL SEARCH REPORT

Inte...al Application No
PCT/CA 93/00522IPC 5 CLASSIFICATION OF SUBJECT MATTER
G01N33/68 G01N33/577 C12P21/08 C12N5/18 C07K7/08
C07K7/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 G01N C07K C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>IMMUNOLOGY vol. 77, 1992, OXFORD, GB pages 609 - 616</p> <p>K. MORGAN ET AL. 'Identification of an immunodominant B-cell epitope in bovine type II collagen and the production of antibodies to type II collagen by immunization with a synthetic peptide representing this epitope' see the whole document</p> <p>---</p> <p>-/-</p>	1-29, 33-35

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *&* document member of the same patent family

2

Date of the actual completion of the international search

18 March 1994

Date of mailing of the international search report

07.04.94

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentzaak 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl
Fax. (+31-70) 340-3016

Authorized officer

Doepfer, K-P

INTERNATIONAL SEARCH REPORT

Int'l Application No
PCT/CA 93/00522

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	<p>MATRIX vol. 13, no. 2, February 1993, STUTTGART, DE pages 95 - 102</p> <p>JOHN S. MORT ET AL. 'Direct Evidence for Active Metalloproteinases Mediating Matrix Degradation in Interleukin 1-stimulated Human Articular Cartilage' cited in the application see page 97, left column, line 40 - right column, line 43</p> <p>---</p>	1-25
A	<p>JOURNAL OF BIOLOGICAL CHEMISTRY vol. 262, no. 23, 15 August 1987, BALTIMORE, MD US pages 11345 - 11350</p> <p>NICHOLAS P. MORRIS AND HANS PETER BÄCHINGER 'Type XI Collagen Is a Heterotrimer with the Composition (1alpha,2alpha,3alpha) Retaining Non-triple-helical Domains' cited in the application see the whole document</p> <p>---</p>	1-25
A	<p>THE JOURNAL OF CLINICAL INVESTIGATION vol. 83, no. 2, February 1989, NEW YORK, NY, US pages 647 - 661</p> <p>GEORGE R. DODGE AND A. ROBIN POOLE 'Immunohistochemical Analysis of Type II Collagen Degradation in Human Normal, Rheumatoid, and Osteoarthritic Cartilages and in Explants of Bovine Articular Cartilage Cultured with Interleukin 1' cited in the application see the whole document</p> <p>---</p>	1-25
A	<p>BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS vol. 118, no. 3, 14 February 1984, DULUTH, MINNESOTA US pages 724 - 729</p> <p>DAVID R. EYRE ET AL. 'All three chains of 1alpha2alpha3alpha collagen from hyaline cartilage resist human collagenase' cited in the application see the whole document</p> <p>---</p>	1-25
2	<p>EP,A,0 505 210 (ORION-YHTYMÄ OY) 23 September 1992 see the whole document</p> <p>---</p> <p>---</p>	1-29

INTERNATIONAL SEARCH REPORT

Interr. Application No.
PCT/CA 93/00522

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>SEMINARS IN ARTHRITIS AND RHEUMATISM vol. 13, no. 1, August 1983, NEW YORK, NY, US pages 1 - 86 MARCEL E. NIMNI 'Collagen: Structure, Function, and Metabolism in Normal and Fibrotic Tissues' cited in the application see page 14, left column, line 4 - page 17, left column, line 45 see page 25, left column, line 33 - page 27, left column, line 34 -----</p>	1-25
2		

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 93/00522

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0505210	23-09-92	NONE	

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P15722PC	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/SE00/01179	International filing date (day/month/year) 07.06.2000	Priority date (day/month/year) 09.06.1999
International Patent Classification (IPC) or national classification and IPC7 G01N 33/53, A61K 39/395, A61K 38/17		
Applicant A+ Science Invest AB et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I Basis of the report
- II Priority
- III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV Lack of unity of invention
- V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI Certain documents cited
- VII Certain defects in the international application
- VIII Certain observations on the international application

Date of submission of the demand 08.01.2001	Date of completion of this report 12.10.2001
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. 08-667 72 88	Authorized officer Carl-Olof Gustafsson/BS Telephone No. 08-782 25 00

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/SE00/01179

I. Basis of the report

1. With regard to the elements of the international application:*

 the international application as originally filed the description:

pages _____, as originally filed

pages _____, filed with the demand

pages _____, filed with the letter of _____

 the claims:

pages _____, as originally filed

pages _____, as amended (together with any statement) under article 19

pages _____, filed with the demand

pages _____, filed with the letter of _____

 the drawings:

pages _____, as originally filed

pages _____, filed with the demand

pages _____, filed with the letter of _____

 the sequence listing part of the description:

pages _____, as originally filed

pages _____, filed with the demand

pages _____, filed with the letter of _____

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language _____ which is:

 the language of a translation furnished for the purposes of international search (under Rule 23.1(b)). the language of publication of the international application (under Rule 48.3(b)). the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

 contained in the international application in written form. filed together with the international application in computer readable form. furnished subsequently to this Authority in written form. furnished subsequently to this Authority in computer readable form. The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the _____ international application as filed has been furnished. The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.4. The amendments have resulted in the cancellation of: the description, pages _____ the claims, Nos. _____ the drawings, sheet/fig _____5. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2 (c)).**

* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

** Any replacement sheet containing such amendments must be referred to under item I and annexed to this report.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/SE00/01179

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

the entire international application,

claims Nos. 5-8

because:

the said international application, or the said claims Nos. 5-8

relate to the following subject matter which does not require an international preliminary examination (*specify*):

See PCT Rule 67.1.(iv).: Methods for treatment of the human or animal body by surgery or therapy as well as diagnostic methods.

the description, claims or drawings (*indicate particular elements below*) or said claims Nos.

are so unclear that no meaningful opinion could be formed (*specify*):

the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

no international search report has been established for said claims Nos. _____

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

the written form has not been furnished or does not comply with the standard.

the computer readable form has not been furnished or does not comply with the standard.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/SE00/01179

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	2-4	YES
	Claims	1	NO
Inventive step (IS)	Claims		YES
	Claims	1-4	NO
Industrial applicability (IA)	Claims	1-4	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The present invention pertains to a kit for diagnosing disc herniation (claim 1) and to the use of antibodies or antigens connected to nucleus pulposus for the manufacture of medicaments for the treatment or diagnosis of disc herniation (claims 2-4).

The wording of claim 1 "antigens from nucleus pulposus" and "antibodies to nucleus pulposus" neither reveals the epitope nor the antigen! In fact, the claim is not even limited to autoantigens/autoantibodies or to antigens or antibodies that are specific for the nucleus pulposus cells. Thus, a person skilled in the art would not be able to select which antigens to use in the kit or realise the different applications referred to in claims 2-8. Consequently, the claims do not fulfil the requirements of clarity and conciseness stated in Art. 6.

The wording "false antibodies" is not a frequently used expression. It seems to correspond to the phrase "blocking antibodies" which is commonly used.

No unified criteria exist in the PCT for assessing the industrial applicability of the subject matter of claims 2-4. Whether the claims are acceptable depend on how the claims are formulated. Some countries allow claims to a known compound for a first use in medical treatment (first medical indication). These countries may also allow claims to the use of such a compound for the manufacture of a medicament for a new medical treatment (second medical indication). The considerations given are based on the acceptance of such claims according to national legislation.

.../...

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Box V

Claims relating to the treatment of a disease may be accepted and examined in some countries. However, owing to differences in national practice and law, it is not possible for the Preliminary Examination Authority to give a valid statement on such claims for all elected states.

The International Search Report revealed four documents considered to be of particular relevance:

- D1 WO9841865 see page 2, 1 14 - p 3, 1 27 and claim 14
- D2 WO9108760 see claims
- D3 WO9702837, see p 3, 1.12 - p 4
- D4 US 5399347, see col. 3 and claims

Document D1 shows that it is known to detect auto-antibodies towards collagen type II or break down products of collagen type II with diagnostic kits. Collagen is a major component of nucleus pulposus. Thus, due to the rather broad wording and the fact that autoantibodies to collagen are likely to be involved in autoimmune reactions to nucleus pulposus, claim 1 would lack novelty. As admitted by the applicant "it is true that collagen is present in the nucleus pulposus". Consequently, "antigens from nucleus pulposus" do indeed include collagenous antigens. Consequently, as the kit according to claim 1 has not been restricted to specific antigens, the kit lacks novelty with regard to the D1 kit for collagen assay.

D2-D4 teach the treatment of autoimmune diseases such as rheumatoid arthritis by administration of autoantigens, e.g. collagen or fragments thereof. In analogy with the above discussion of D1, it would seem obvious to use similar antigens in the manufacture of a medicament for the treatment of any collagen degenerative disease. Provided that the claims can be restricted to autoantigens (autoantibodies or corresponding idiotypic antibodies) that are unique to nucleus pulposus, a redrafted claim 4 would fulfil the requirements of novelty and inventive step.

Degenerative diseases within the nucleus pulposus have been postulated to be related to autoimmune mechanisms. However, these early findings seem not to have led to diagnostics or remedies for disc herniation. Consequently, no immediate conclusions can be drawn from this knowledge

.../...

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Internal application No.

PCT/SE00/01179

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Box V

To the extent that claims 2-4 could be amended to satisfy the requirement of clarity and conciseness discussed above, these claims may fulfil the requirements of novelty, inventive step and industrial applicability for countries that accept second medical indication-claims.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
14 December 2000 (14.12.2000)

PCT

(10) International Publication Number
WO 00/75659 A1

(51) International Patent Classification⁷: G01N 33/53,
A61K 39/395, 38/17

CN, CR, CU, CZ, CZ (utility model), DE, DE (utility model), DK, DK (utility model), DM, DZ, EE, EE (utility model), ES, FI, FI (utility model), GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KR (utility model), KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (utility model), SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(21) International Application Number: PCT/SE00/01179

(22) International Filing Date: 7 June 2000 (07.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9902155-2 9 June 1999 (09.06.1999) SE

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(71) Applicants and

(72) Inventors: OLMARKER, Kjell [SE/SE]; Gustavsgatan
35, S-431 66 Mölndal (SE). RYDEVIK, Björn [SE/SE];
Laboratorietrappan 6, S-412 68 Göteborg (SE).

(74) Agent: GÖTEBORGS PATENTBYRÅ DAHLS AB;
Sjöporten 4, S-417 64 Göteborg (SE).

(81) Designated States (national): AE, AG, AL, AM, AT, AT
(utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH,

Published:

- With international search report.
- Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/75659 A1

(54) Title: ANTIBODIES TO NUCLEUS PULPOSUS IN DISC HERNIATION, DIAGNOSTIC KIT, MEDICAL PREPARATIONS AND TREATMENT

(57) Abstract: The present invention relates to antibodies towards nucleus pulposus cells and the use thereof for manufacturing a kit for diagnosing disc herniation, as well as anti-antibody to said antibody, and its use for treating disc herniation as well as sciatica, as well as a false antibody to the antibody to nucleus pulposus cells as well as soluble antigens from nucleus pulposus cells to block such said antibodies.

TITLE

ANTIBODIES TO NUCLEUS PULPOSUS IN DISC HERNIATION,
DIAGNOSTIC KIT, MEDICAL PREPARATIONS AND TREATMENT

DESCRIPTION

5 Technical field

The present invention relates the use of serum antibodies for the diagnosis and treatment of disc herniation with resulting nerve root pain in the cervical and lumbar spine such as sciatica.

10 The object of the present invention is to obtain improved methods in diagnosis and treatment of nerve root pain such as sciatica and other radiculopathies related to disc herniation in the cervical or lumbar spine.

Background of the invention

15 The exact pathophysiological mechanisms leading to sciatica in relation to herniation of intervertebral discs are not fully understood. Recently it was demonstrated that the nucleus pulposus (the viscous component of the intervertebral disc that leaks out into the spinal canal in case of disc herniation) may induce structural and functional changes in the adjacent nerve root (1-14). Also, it has been shown that nerve roots experimentally exposed
20 to nucleus pulposus become sensitive to mechanical deformation thereby producing pain (8,13). Certain pro-inflammatory cytokines, produced by the nucleus pulposus cells, have been defined as being responsible for inducing these effects (10). However, there is both clinical and experimental evidence which may suggest that also immunologic mechanisms may be present to a certain extent. It has been suggested that, since the nucleus pulposus is
25 secluded from the immune-system from birth, being a non-vascularized tissue, the immune system has not regarded the nucleus pulposus as "self" during early embryonic stages, but would instead consider the nucleus pulposus as "non-self" later in life (15-22). At disc herniation, possible antigens in the nucleus pulposus might thus be presented to the immune system and there would be an auto-immune reaction induced towards these antigens. The
30 reaction would mainly involve the nucleus pulposus, but the substances might also induce changes in the adjacent nerve roots secondary to this reaction. These suggested substances would most likely be the same pro-inflammatory cytokines as previously being defined as

inducing nerve root injury. There is reason to believe that such mechanisms also relates to radiculopathies in the upper extremities due to disc herniation in the cervical spine. However, no one has previously been able to demonstrate the presence of antibodies in serum towards the nucleus pulposus of the same individual.

5

In order to isolate and show the presence of antibodies towards nucleus pulposus cells the following experiments and tests were conducted.

Material and methods

10 1) Culture of nucleus pulposus cells:

One pig weighing 26 kg was anaesthetized with an intra muscular injection of 20 mg/kg body weight of Ketalar^R (ketamine 50 mg/ml, Parke-Davis, Morris Plains, NJ) and an intravenous injection of 4 mg/kg body weight of Hypnodil^R (methomidate chloride 50 mg/ml, AB Leo, Helsingborg, Sweden) and 0,1 mg/kg body weight of Stresnil^R (azaperon, 2 mg/ml, Janssen Pharmaceutica, Beerse, Belgium).

15 Approximately 20 ml of blood were collected and allowed to coagulate at room temperature. It was then centrifuged and the supernatant (serum) was stored at 80°C in a refrigerator.

20

After induction of anaesthesia, the pig was killed by an overdose of potassium chloride. The lumbar and lower part of the thoracic spine was removed en bloc. The spine was cleansed from muscles and tendons. Under sterile conditions the discs were incised and the nucleus pulposus was harvested. The nucleus pulposus (NP) was washed once in Ham's F12

25 medium (Gibco BRL, Paisley, Scotland). The NP from discs were placed in a test tube with Ham's F12 medium and centrifuged. The remaining pellet was dissolved in 6 ml of Ham's F12 with 3 ml of trypsin 2.5 % in a 75 cm² culture flask for 30 minutes at 37°C. Then 6 ml of Ham's F12 with 12 mg of collagenase (Sigma Cat. No. C9407) were added. After 3.5 hrs at 37°C the content of the culture flask was transferred to a test tube and centrifuged. The 30 separated NP-cell pellets were suspended in DMEM/F12 1:1 medium (Gibco BRL, Paisley, Scotland) supplemented with 1% L-glutamine (200 mM, Gibco BRL, Paisley, Scotland), 50 µg/ml gentamycin sulphate (Gibco BRL, Paisley, Scotland) and 10% foetal calf serum

(FCS, Gibco BRL, Paisley, Scotland). Fungizone 2 μ g/ml and α -ascorbic acid 50 μ g/ml was added. The cells were cultured in 25 cm^2 flasks (Costar, Cambridge, MA), at 37°C and 5% CO₂ in air for 3-4 weeks. After 2 weeks the cells were transferred to 4-chamber polystyrene vessel tissue culture treated glass slides (Becton Dickinson Labware, Franklin Lakes, NJ).

5 Following 3 days of culture the slides were used for the assessment as will be described below.

2) Culture of fibroblasts

A 2x2 cm big piece of the skin was harvested at the same time as the nucleus pulposus 10 under sterile conditions. The dermis of the skin was cut in small pieces and put in spinner bottles with 10 ml of collagenase solution (0.8 mg/ml, Sigma Chemical, St. Louis, MO, in Ham's F12 medium) for 90 minutes in 37°C water bath. The separated fibroblasts were centrifuged and transferred to 75 cm^2 tissue culture flasks (Costar, Cambridge, MA), with DMEM/F12 1:1 medium supplemented as above for NP-cells.

15

3) Pretreatment of the serum

The cultured fibroblasts were liberated from the culture flasks by treatment of 0.125% trypsin solution (Gibco BRL, Paisley, Scotland) and added to the serum. The addition of 20 fibroblasts was performed in order to eliminate the risk that antibodies in the serum which non-specifically would bind to cultured cells, would be applied to the nucleus pulposus cells. The test-tube was centrifuged and the supernatant collected (serum with remaining antibodies).

4). Assessment of the presence of antibodies in serum towards autologous nucleus pulposus

25 cells

The culture slides with the cultured nucleus pulposus cells were fixed in acetone for 10 minutes and then dried in air. The slides were washed twice for 5 minutes in PBS (Phosphate Buffered Saline, Life Technologies Ltd., Paisley, Scotland) The slides were then treated with 0.3% H₂O₂ (Sigma Chemical, St. Louis, MO) for 30 minutes and then washed 30 twice for 5 minutes in PBS. The slides were then exposed to standard freeze-dried milk (5% in PBS) for 30 minutes to block irrelevant antigens, and then washed twice for 5 minutes in PBS.

The cultured NP-cells were exposed to

- a) one drop of the pretreated serum,
- b) one drop of the pretreated serum diluted by PBS 1:40; or
- c) not in serum at all, and

5 incubated for 1 hr at room temperature, and then washed twice for 5 minutes in PBS. The culture slides were then incubated with the secondary antibody (Peroxidase-Conjugated Rabbit Anti-Swine immunoglobulin, Code No. P164, Dako A/S, Glostrup, Denmark) for 30 minutes, and then washed twice for 5 minutes in PBS. The slides were finally developed with DAB (3,3'-diaminobenzidine, 10 mg in 5 ml PBS, and 17 μ l H₂O₂ (3%), Sigma 10 Chemical, St. Louis, MO) for 2 minutes, and then washed twice for 5 minutes in PBS. The specimens were dehydrated in a series of alcohol-dilutions and assessed by light microscopy.

Results

15 a) Pretreated serum

There was a clear staining of the cell membranes of the nucleus pulposus cells and also of the nuclei of the cells. This indicates the presence of specific antibodies towards the nucleus pulposus cells in serum from the same individual (autologous).

20 b) Pretreated serum at 1:40

There was a similar staining of the cells as for the concentrated serum, although not as pronounced.

c) Nucleus pulposus cells not exposed to serum

25 There was no staining of the cells and the cells were difficult to distinguish on the culture slides. This suggests that the secondary antibody (rabbit:anti-swine-immunoglobulin) did not non-specifically bind to the pig nucleus pulposus cells and that the staining of a) and b) was the result of the addition of specific antibodies from the serum.

30 Conclusion and comments

The following conclusion can be made from the present experiment:

- There are antibodies present in serum that specifically bind to nucleus pulposus cells of the

same individual

The following comments can be made from the present experiment

- From this study it can not be recognized if the antibodies are readily available in high

5 concentrations in serum or if there had been an immunization to the nucleus pulposus in the pig. If there are antibodies already present in the serum, the antigen may be a potent antigen, comparable for instance to the MHC (Major Histocompatibility Complex) antigens.

- Regardless of the nature of the antigen one can suspect that the levels in serum may increase the levels of these antibodies in case of disc herniation and sciatica and therefore

10 used as a diagnostic tool.

- At present the diagnosis of sciatica is made by patient history and radiologic findings.

However, since it is known that almost 30% of the population without any complaints of sciatica also have disc herniations at radiological examinations, the radiologic diagnosis is less valuable (23-25). It has been suggested that disc herniations can be either active

15 (symptoms) or inactive (no symptoms). Based on the findings in the present study it is assumed that an active disc herniation is related to inflammatory and immunologic changes, whereas the inactive disc herniation is a mere protrusion of disc tissue without triggering of the immune system. The lack of immunologic reaction might be based on either the nucleus pulposus still being isolated from the epidural space by remaining membranes or a less 20 developed immunoreactivity of the patient, alternatively lack of sensitizing antigens in the disc cells.

The present invention can thus be used in the form of an antigen containing diagnostic kit for diagnosing disc herniation, in particular disc herniation leading to sciatica. Further the

25 effects of serum antibodies towards the nucleus pulposus cells (NP-antibodies) can be neutralized in three ways. First the NP-antibodies can be inactivated by administering a specific antibody for such serum antibodies, an anti-antibody. Secondly, the effects of the NP-antibodies can be inhibited by administering a substance that is similar to the NP-antibody, a false antibody, and binds to the antigen in the nucleus pulposus in stead of the 30 antibody, which false antibody is able to bind to and block the antigen in such a way that an immunological reaction is inhibited. Thirdly, soluble antigens corresponding to the NP-antibodies can be administered, thereby blocking the effects of the NP-antibodies. In such

ways the action of the NP-antibodies can be blocked since the NP-antibodies are prevented from binding to its antigen.

The compounds of the invention can be administered in a variety of dosage forms, e.g.,

5 orally, in the form of tablets, capsules, sugar or film coated tablets, liquid solutions; rectally, in the form of suppositories; parenterally, e.g., intramuscularly or by intravenous injection or infusion. The therapeutic regimen for the different clinical syndromes must be adapted to the type of pathology taken in to account, as usual, also the route of administration, the form in which the compound is administered and age, weight, and condition of the subject

10 involved.

The oral route is employed, in general, for all conditions, requiring such compounds. In emergency cases preference is given to intravenous injection. For these purposes the compounds of the invention can be administered orally at doses ranging from about 20 to

15 about 1500 mg/day. Of course, these dosage regimens may be adjusted to provide the optimal therapeutic response.

The nature of the pharmaceutical composition containing the compounds of the invention in association with pharmaceutically acceptable carriers or diluents will, of course, depend

20 upon the desired route of administration. The composition may be formulated in the conventional manner with the usual ingredients. For example, the compounds of the invention may be administered in the form of aqueous or oily solutions or suspensions, tablets, pills, gelatine capsules (hard or soft ones)syrups, drops or suppositories.

25 Thus for oral administration, the pharmaceutical compositions containing the compounds of the invention are preferably tablets, pills or gelatine capsules, which contain the active substance together with diluents, such as lactose, dextrose, sucrose, mannitol, sorbitol, cellulose; lubricants, e.g., silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols; or they may also contain binders, such as starches, gelatine, methyl

30 cellulose, carboxymethylcellulose, gum arabic, tragacanth, polyvinylpyrrolidone; disaggregating agents such as starches, alginic acid, alginates, sodium starch glycolate, microcrystalline cellulose; effervescing agents such a carbonates and acids; dyestoffs;

sweeteners; wetting agents, such as lecithin, polysorbates, laurylsulphates; and in general non-toxic and pharmaceutically inert substances used in the formulation of pharmaceutical compositions. The mentioned pharmaceutical compositions may be manufactured in known manners, e.g., by means of mixing, granulating, tableting, sugar-coating or film-coating 5 processes. In the case film providing compounds can be selected to provide release in the right place in the intestinal tract with regard to absorption and maximum effect. Thus pH-dependent film formers can be used to allow absorption in the intestines as such, whereby different phthalate are normally used or acrylic acid/methacrylic acid derivatives and polymers.

10

The liquid dispersions for oral administration may be e.g., syrups, emulsion, and suspensions.

15 The syrups may contain as carrier, e.g., saccharose, or saccharose with glycerine and/or mannitol and/or sorbitol.

Suspensions and emulsions may contain as carrier, e.g., a natural gum, such as gum arabic, xanthan gum, agar, sodium alginate, pectin, methyl cellulose, carboxymethylcellulose, polyvinyl alcohol.

20

The suspension or solutions for intramuscular injections may contain together with the active compound, a pharmaceutically acceptable carrier, such as e.g., sterile water, olive oil, ethyl oleate, glycols, e.g., propylene glycol, and if so desired, a suitable amount of lidocaine hydrochloride. Adjuvants for triggering the injection effect can be added as well.

25

The solutions for intravenous injection or infusion may contain as carrier, e.g., sterile water, or preferably, a sterile isotonic saline solution, as well as adjuvants used in the field of injection of active compounds.

30 The suppositories may contain together with the active compound, a pharmaceutically acceptable carrier, e.g., cocoa-butter polyethylene glycol, a polyethylene sorbitan fatty acid ester surfactant or lecithin.

REFERENCES

1. Olmarker K, Rydevik B, Nordborg C. Autologous nucleus pulposus induces neurophysiologic and histologic changes in porcine cauda equina nerve roots [see comments]. *Spine* 1993;18:1425-32.
- 5 2. Olmarker K, Byrod G, Comefjord M, Nordborg C, Rydevik B. Effects of methylprednisolone on nucleus pulposus-induced nerve root injury. *Spine* 1994; 19:1803-8.
3. Olmarker K, Nordborg C, Larsson K, Rydevik B. Ultrastructural changes in spinal nerve roots induced by autologous nucleus pulposus. *Spine* 1996;21:411-4.
4. Kayama S, Konno S, Olmarker K, Yabuki S, Kikuchi S. Incision of the anulus fibrosis induces nerve root morphologic, vascular, and functional changes. An experimental study. *Spine* 1996;21:2539-43.
- 10 5. Olmarker K, Brisby H, Yabuki S, Nordborg C, Rydevik B. The effects of normal, frozen, and hyaluronidase-digested nucleus pulposus on nerve root structure and function. *Spine* 1997;22:4715; discussion 476.
- 15 6. Otani, K, Kikuchi, S, Arai, I, Mao GP, Konno, S, Olmarker, K: Experimental disc herniation. Evaluation of the natural course using assessment of changes in nerve conduction of the spinal nerve roots and MRI-changes of the intervertebral discs. *Spine* 22:24, 2894-90, 1997
7. Kayama S, Olmarker K, Larsson K, Sjögren-Jansson E, Lindahl A, Rydevik B. Cultured, 20 autologous nucleus pulposus cells induce structural and functional changes in spinal nerve roots. *Spine* 23:20, 2155-8, 1998
8. Olmarker K, Myers RR. Pathogenesis of sciatic pain: Role of herniated nucleus pulposus and deformation of spinal nerve root and DRG. *PAIN*, 78:9, 105, 1998
9. Yabuki S, Kawaguchi Y, Olmarker K, Rydevik B. Effects of lidocaine on nucleus 25 pulposus-induced nerve root injury. *Spine*, 23:22, 2383-9, 1998
10. Olmarker, K, Larsoon, K:TNFa and nucleus pulposus-induced nerve root injury. *Spine* 23:23, 2538-44, 1998
11. Byrđ, G, Olmarker, K, Nordborg, C, Rydevik, B: Early effects of nucleus pulposus application on spinal nerve root morphology and function. *Eur Spine J.* 7, 445-49, 1998
- 30 12. Yabuki, S, Kikuchi, S, Olmarker, K, Myers RR: Acute effects of nucleus pulposus on blood flow and endoneurial fluid pressure in rat dorsal root ganglia. *Spine* 23:23, 2517-23, 1998.

13. Olmarker K, Iwabuchi M, Larsson K, Rydevik B. Effects of in vitro degenerated nucleus pulposus on nerve root conduction velocity. *Eur Spine Journal*, 7:5, 394-9, 1998
14. Otani K, Mao GP, Arai I, Konno S, Olmarker K, Kikuchi S. Nucleus pulposus-induced increase in vascular permeability in the nerve root. *Manuscript*
- 5 15. Bobechko, WP, Hirsch, C: Auto-immune response to nucleus pulposus in the rabbit. *J Bone Joint Surg.* 47 B 3, 574-580, 1965
16. LaRocca, H: New horizons in research in disc disease. *Orthop Clin N Am*, 2, 521, 1971
17. Naylor, A: Biochemical changes in human intervertebral disc degeneration and prolapse. *Orthop Clin N Am*, 2:2, 343, 1971
- 10 18. Gertzbein, SD, Tile, M, Gross, A, Falk, R: Autoimmunity in degenerative disease of the lumbar spine. *Orthop Clin N Am*, 6:1, 67-73, 1975
19. Bisla, RS, Marchisello, PJ, Lockshin, MD, Hart, DM, Marcus, RE, Granda, J: Autoimmunological basis of disc degeneration. *Clin Orthop*, 123, 149-154, 1976
20. Gertzbein, SD: Degenerative disc disease of the lumbar spine. Immunological implications. *Clin Orthop*, 129:69-71, 1977
- 15 21. Gertzbein, SD, Tait JH, Devlin, SR: The stimulation of lymphocytes by nucleus pulposus in patients with degenerative disc disease of the lumbar spine. *Clin Orthop*, 123, 149-154, 1977
22. Olmarker, K: Experimental basis of sciatica, *J Orthop Science*, 1, 230-42, 1996
- 20 23. Boden, SD, Davis, DO, Dina, TS, Patronas, NJ, Wiesel, SW: Abnormal magnetic-resonance scans of the lumbar spine in symptomatic subjects. A prospective investigation. *J Bone Joint Surg*, 72:3, 403-8, 1990
24. Boos, N, Rieder, R, Schade, V, Spratt, KF, Semmer, N, Aebi, M: The diagnostic accuracy of magnetic resonance imaging, work perception and psychosocial factors in identifying symptomatic disc herniations. *Spine*, 20:24, 2613-25, 1995
- 25 25. Weishaupt, D, Zanetti, M, Hodler, J, Boos, N: MR imaging of the lumbar spine: prevalence of intervertebral disc extrusion and sequestration, nerve root compression, end plate abnormalities, and osteoarthritis of the facet joints in asymptomatic volunteers. *Radiology*, 209:3, 661-6, 1998.
- 30 26. Satoh, K, Konno, S, Nishiyama, K, Olmarker, K, Kikuchi, S: Presence and distribution of antigen-antibody complexes in the herniated nucleus pulposus. *Submitted*.

CLAIMS

1. Kit for diagnosing disc herniation,

characterized in that it comprises antigens from nucleus pulposus cells for determining an optional presence of antibodies to nucleus pulposus.

5

2. The use of an anti-antibody to antibodies to nucleus pulposus cells in the manufacture of a medicament for the treatment of disc herniation.

3. The use of a false antibody to nucleus pulposus cells in the manufacture of a medicament

10 for the treatment of disc herniation, which false antibody is able to bind to and block the antigen in such a way that an immunological reaction is inhibited.

4. The use of soluble antigens from nucleus pulposus cells in the manufacture of a medicament or a diagnostic means for the diagnosis or treatment of disc herniation.

15

5. Method for treating disc herniation, whereby a therapeutically efficient amount of a compound that prevents the binding of serum antibodies to nucleus pulposus cells to bind to nucleus pulposus.

20 6. Method for treating disc herniation, whereby a therapeutically efficient amount of an anti-antibody to antibodies of nucleus pulposus cells is administered.

7. Method for treating disc herniation, whereby a therapeutically efficient amount of a false antibody to nucleus pulposus is administered, which false antibody is able to bind to and block the antigen in such a way that an immunological reaction is inhibited.

25 8. Method for treating disc herniation, whereby a therapeutically efficient amount of soluble antigens from nucleus pulposus cells is administered.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 00/01179

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: G01N 33/53, A61K 39/395, A61K 38/17
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61K, G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE, DK, FI, NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9841865 A1 (MONTECH MEDICAL DEVELOPMENT PTY. LTD. ET AL), 24 Sept 1998 (24.09.98), page 2, line 14 - page 3, line 27, claim 14 --	1
A	WO 9414070 A1 (SHRINERS HOSPITALS FOR CRIPPLED CHILDREN), 23 June 1994 (23.06.94), page 11, line 24 - line 37; page 50 - page 61, claims 17-25 --	1
X	WO 9108760 A1 (BRIGHAM AND WOMEN'S HOSPITAL ET AL), 27 June 1991 (27.06.91) --	4,5,8
A	US 4628027 A (STEFFEN GAY), 9 December 1986 (09.12.86), column 6, line 56 - column 7, line 46 --	1

Further documents are listed in the continuation of Box C.

See patent family annex.

- * Special categories of cited documents
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

25 Sept 2000

03-10-2000

Name and mailing address of the ISA/
Swedish Patent Office
Box 5055, S-102 42 STOCKHOLM
Facsimile No. +46 8 666 02 86

Authorized officer
Carl-Olof Gustafsson/GH
Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 00/01179

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9702837 A1 (AUROIMMUNE, INC.), 30 January 1997 (30.01.97), page 3, line 12 - page 4 --	4,5,8
X	US 5399347 A (DAVID E. TRENTHAM ET AL), 21 March 1995 (21.03.95), column 3, claims --	4,5,8
A	Clinical orthopaedics and related research, Volume 129, 1977, S.D.Gertzbein, M.D., "Degenerative Disk Disease of the Lumbar Spine: Immunological Implications", page 68 - page 71, see page 70 --	1-8
A	US 5266564 A (MANUEL MODOLELL ET AL), 30 November 1993 (30.11.93), column 3 -----	1-8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE00/01179

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: 5-8
because they relate to subject matter not required to be searched by this Authority, namely:

see extra sheet
2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).:

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see extra sheet

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE00/01179

Claims 5-8 relate to methods of treatment of the human or animal body by surgery or by therapy/diagnostic methods practised on the human or animal body/Rule 39.1.(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the compounds/compositions.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE00/01179

The claims refer to a kit for diagnosing disc herniation (claim 1) and to 3 a posteriori independent uses of antibodies or antigens connected to nucleus pulposus for the manufacture of medicaments for the treatment of disc herniation or for diagnosis of disc herniation (claims 2-4); compare WO9841865, see claims 14 and p 2-3 and WO941470, claims 17-25 and p 50-61.

1. Kit for diagnosis of disc herniation comprising antigens from nucleus pulposus (NP) for determining antibodies to NP according to claim 1.
2. Use of an anti-antibody to antibodies to NP in the manufacture of a medicament for the treatment of disc herniation according to claim 2
3. Use of a "false antibody" to NP in the manufacture of a medicament for the treatment of disc herniation according to claim 3.
4. Use of a soluble antigen from NP in the manufacture of a medicament for the treatment of disc herniation or as a diagnostic means for diagnosis of disc herniation according to claim 4.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.	
PCT/SE 00/01179	

Patent document cited in search report	Publication date	Patent family members	Publication date
WO 9841865 A1	24/09/98	AU 698 A AU P051297 D ZA 9802338 A	12/10/98 00/00/00 08/10/98
WO 9414070 A1	23/06/94	AU 5558394 A	04/07/94
WO 9108760 A1	27/06/91	AT 190496 T AU 642693 B AU 6979191 A BR 9007950 A CA 2070281 A DE 69033487 D, T EP 0506785 A, B SE 0506785 T3 ES 2144398 T HU 61487 A HU 210715 B HU 9202072 D IL 96734 A JP 2625253 B KR 185747 B US 5571499 A US 5571500 A US 5641473 A US 5641474 A US 5645820 A US 5720955 A US 5783188 A US 5843445 A	15/04/00 28/10/93 18/07/91 27/10/92 21/06/91 29/06/00 07/10/92 16/06/00 28/01/93 28/06/95 00/00/00 14/11/96 02/07/97 01/05/99 05/11/96 05/11/96 24/06/97 24/06/97 08/07/97 24/02/98 21/07/98 01/12/98
US 4628027 A	09/12/86	AU 1703683 A CA 1251729 A EP 0109434 A ES 522508 A ES 8501630 A GR 78570 A MX 197328 A NZ 204255 A WO 8304104 A ZA 8303483 A	02/12/83 28/03/89 30/05/84 01/12/84 01/03/85 27/09/84 28/02/94 08/10/86 24/11/83 27/06/84
WO 9702837 A1	30/01/97	AU 6491596 A CA 2235718 A EP 0837693 A IL 122824 D NO 976034 A US 5856446 A	10/02/97 30/01/97 29/04/98 00/00/00 03/02/98 05/01/99

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SE 00/01179

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5399347 A	21/03/95	AU 5439994 A	26/04/94
		EP 0662838 A	19/07/95
		IL 107050 D	00/00/00
		JP 8502071 T	05/03/96
		US 5720955 A	24/02/98
		US 5783188 A	21/07/98
		US 5843445 A	01/12/98
		WO 9407520 A	14/04/94
		US 5733547 A	31/03/98
		US 5849298 A	15/12/98
		US 5858364 A	12/01/99
		US 5869054 A	09/02/99
		US 5869093 A	09/02/99
		AU 693232 B	25/06/98
		AU 3040995 A	30/11/95
		AU 9023791 A	20/05/92
		CA 2092905 A	16/04/92
		EP 0553291 A	04/08/93
		HU 69942 A	28/09/95
		HU 9301089 D	00/00/00
		IL 99754 A	04/08/96
		JP 2635444 B	30/07/97
		KR 140841 B	01/06/98
		NO 931372 A	18/05/93
		US 6019971 A	01/02/00
		WO 9206708 A	30/04/92
		AT 130762 T	15/12/95
		AU 632991 B	21/01/93
		AU 2079788 A	19/01/89
		CA 1336954 A	12/09/95
		DE 3854741 D, T	18/04/96
		DK 651689 A	26/02/90
		EP 0359783 A, B	28/03/90
		SE 0359783 T3	
		EP 0666080 A	09/08/95
		JP 2503919 T	15/11/90
		JP 2512796 B	03/07/96
		US 5571499 A	05/11/96
		US 5571500 A	05/11/96
		US 5641473 A	24/06/97
		US 5641474 A	24/06/97
		US 5645820 A	08/07/97
		US 6039947 A	21/03/00
		WO 8810120 A	29/12/88

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SE 00/01179

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5266564 A	30/11/93	AT 94757 T	15/10/93
		AU 5670690 A	07/01/91
		CA 2056421 A	03/12/90
		CS 276439 B	13/05/92
		CS 9002689 A	15/01/92
		DE 3918082 A,C	24/01/91
		DE 59002863 D	00/00/00
		DK 474712 T	29/11/93
		EP 0474712 A,B	18/03/92
		SE 0474712 T3	
		ES 2060173 T	16/11/94
		GR 1001195 B	07/06/93
		GR 90100406 A	15/11/91
		IE 64039 B	28/06/95
		JP 2977894 B	15/11/99
		JP 4507096 T	10/12/92
		WO 9014829 A	13/12/90
		ZA 9004196 A	27/03/91